Bacterial clearance, heterophil function, and hematological parameters of transport-stressed turkey poults supplemented with dietary yeast extract[1]

G. R. Huff,*[2] W. E. Huff,* M. B. Farnell,† N. C. Rath,* F. Solis de los Santos,‡ and A. M. Donoghue*

*USDA, Agricultural Research Service, Poultry Production and Product Safety Research Unit, Fayetteville, AR 72701; †Poultry Science Department, Texas A&M University, College Station 77843; and ‡Animal Science Department, Instituto Superior de Agricultura (ISA), Apartado Postal 166, Santiago, Dominican Republic

ABSTRACT Yeast extracts (YE) contain biological response modifiers that may be useful as alternatives to antibiotics for controlling pathogens in poultry production and mitigating the deleterious effects of production stressors. The objective of the present study was to determine the ability of a commercial dietary YE (Alphamune) to modulate the immune response in male turkey poults challenged with *Escherichia coli* and subjected to transport stress. Alphamune was added to turkey poult diets at 0, 500, or 1,000 g/ton. Poults were challenged by air sac injection with 60 cfu of *E. coli* at 1 wk of age. At 3 wk of age, these challenged birds were subjected to transport stress and birds were bled and necropsied the following morning. Blood cell numbers and percentages, hematological parameters, and clinical chemistry values were determined. Oxidative burst activity of isolated heterophils was measured using stimulation with phorbol myristate acetate and a 2′,7′-dichlorofluorescein diacetate assay. Data were analyzed using GLM and least squares means procedures of the SAS program. The numbers and percentages of heterophils in peripheral blood were increased and their oxidative burst activity was stimulated by YE. The stress challenge dramatically increased oxidative burst and this increase was significantly modulated by YE treatment. Serum levels of calcium, phosphorus, and triglycerides were decreased and uric acid levels, erythrocyte numbers, hemoglobin, and hematocrit were increased by YE supplementation. Bacteria were isolated from the air sac and liver of a lower percentage of birds provided with YE. These results suggest that dietary YE has potential as a nonantibiotic alternative for decreasing bacterial pathogens in turkey production.

Key words: turkey, yeast extract, *Escherichia coli*, transport stress, heterophil

2010 Poultry Science 89:447–456
doi:10.3382/ps.2009-00328

INTRODUCTION

Yeast cell walls are potential immunomodulators that may serve as alternatives to antibiotics for both growth promotion and disease resistance in poultry production. Brewer's dried yeast (*Saccharomyces cerevisiae)* products, which are often by-products of beer manufacturing, have been added to animal feeds for many years for their nutritional content because they contain high levels of protein and B vitamins (Stone, 1998). More recently brewer's yeast has been used as a source of cell wall fractions, both mannanoligosaccharides (**MOS**) and β-1,3/1,6-glucan, by several companies providing antibiotic-replacement products for animal production. β-1,3/1,6-Glucans are characterized as biological response modifiers and have been shown to both stimulate and suppress immunity (Leung et al., 2006; Volman et al., 2008; Novak and Vetvicka, 2009; Soltanian et al., 2009). Mannanoligosaccharides have been shown to also have immunomodulating effects in addition to the ability to bind and eliminate pathogenic bacteria with type I fimbriae and to improve gut morphology and health (Yang et al., 2009).

Whole yeast products or yeast cell wall components have been used to improve growth and affect the physiology, morphology, and microbiology of the intestinal tract of both turkey poults (Bradley et al., 1994; Hooge, 2004b; Sims et al., 2004; Zdunczyk et al., 2004, 2005; Huff et al., 2007; Rosen, 2007b; Solis de los Santos, 2007) and broiler chicks (Hooge, 2004a; Zhang et al., 2005; Huff et al., 2006; Rosen, 2007a; Yang et al., 2008a,b; Morales-Lopez et al., 2009). These products

Received July 8, 2009.
Accepted October 24, 2009.
[1]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.
[2]Corresponding author: grhuff@uark.edu

have also been shown to affect immunity in both chickens and turkeys (Guo et al., 2003; Cetin et al., 2005; Lowry et al., 2005; Chae et al., 2006; Chen et al., 2008; Gomez-Verduzco et al., 2009).

For many years, growth-promoting and therapeutic antibiotics have been successfully used to compensate for the potential stressors that can be present in intensive animal production. Although growth-promoting antibiotics are thought to function mainly by changing the intestinal bacterial flora and affecting gut development (Dibner and Richards, 2005), another mechanism by which they may improve production values is through their ability to modulate the inflammatory response to subclinical disease caused by opportunistic pathogens and their concomitant molecules that are ubiquitous in the environment, such as *Escherichia coli* and the lipopolysacharide component of its cell wall (Roura et al., 1992). The potential stressors of intensive poultry production can lead to changes in the immune response that make animals more susceptible to these pathogens and thus lead to disease. Because stress can have both positive and negative effects on growth, feed conversion, and immune function in poultry (Siegel, 1995), the results of many stress experiments are equivocal. Our research program, using an *E. coli* respiratory disease challenge model, has allowed us to study the effects of different stressors on disease and develop nutritional strategies for increasing both disease resistance and production values in turkeys and broiler chickens (Huff et al., 2000, 2004, 2006).

The objective of the present study was to determine the ability of a commercial dietary yeast extract (Alphamune, Alpharma Animal Health, Antwerp, Belgium) that combines a standardized level of 1,3/1,6 β-glucan with the performance enhancement of MOS, to modulate the immune response in young turkey poults challenged with *E. coli* and subjected to transport stress.

MATERIALS AND METHODS

Day-of-hatch male Hybrid Converter poults were obtained from a commercial hatchery and placed in battery brooders. Pen placement was completely randomized in a 3 × 2 feed treatment × stress challenge experimental design with 3 pens containing 10 birds/pen in each treatment × challenge group. Birds were provided ad libitum access to water and an unmedicated standard corn (46.5%) and soybean (41%) turkey starter diet that met or exceeded the NRC recommended allowances (NRC, 1994) or the same diet supplemented with 500 g/ton or 1,000 g/ton of Alphamune yeast extract (**YE**). The basal diet contained 2,884 kcal of ME/kg and 28.8% CP. Body weights and feed conversion efficiency were determined weekly.

Stress Challenge Treatment

At 1 wk of age, the challenged group of poults was inoculated in the left cranial-thoracic air sac with sterile tryptose phosphate broth (**TPB**) containing approximately 60 cfu of a nonmotile strain of *E. coli* serotype O2, which had originally been isolated from chickens with colisepticemia. The inoculum was prepared by adding 2 inoculating loops of an overnight culture on blood agar to 100 mL of TPB and incubating for 2.5 h in a 37°C shaking water bath. The culture was held overnight at 4°C while a standard plate count was made. Ten-fold dilutions were then made in TPB based on the standard plate count and the challenge dilution titer was verified with another plate count.

At 3 wk of age, these same challenged birds were also subjected to a transport stress protocol. Birds were placed in coops and driven for 3 h, then held in the same coops for 9 h, giving a total of 12 h of containment without feed or water. Birds were returned to their original pens and provided feed and water. Nonchallenged treatment controls were neither stressed nor inoculated.

Bleeding and Necropsy

The morning after transport stress, 9 birds from each experimental group (3 birds/pen) were bled by cardiac puncture and all birds were weighed and necropsied. Total leukocyte counts, erythrocyte numbers, hemoglobin, hematocrit, and the numbers and percentage of heterophils (**HET**), lymphocytes (**LYM**), monocytes (**MONO**), eosinophils, and basophils were determined using a Cell-Dyn 3500 blood analysis system (Abbott Diagnostics, Abbott Park, IL), which employs both electronic impedance and laser light scattering and has been standardized for analysis of turkey blood. Heterophil:lymphocyte ratios (**HET:LYM**), an indicator of stress in birds (Gross and Siegel, 1983), were calculated by dividing the number of HET in 1 mL of peripheral blood by the number of LYM.

The 9 blood samples obtained from each group of stressed and nonstressed control diet-fed birds and from each group of those fed diets supplemented with 1,000 g/ton of YE were pooled and HET were isolated using a 1.077/1.119 Histopaque gradient (Sigma-Aldrich, St. Louis, MO) as described previously (Farnell et al., 2006). Oxidative activity of HET was measured using a Cytofluor 2300 fluorescent plate reader (Millipore Corp., Bedford, MA) and an indicator of reactive oxygen species, 2′,7′-dichlorofluorescein diacetate assay (Molecular Probes Inc., Eugene, OR), as described previously (Xie et al., 2002). Heterophils (2×10^7/mL) were incubated for 30 min with the HET agonist phorbol-12-myristate-13-acetate (**PMA**, 2 g/mL of HET, Calbiochem, La Jolla, CA) at 42°C in a heated orbital shaker plate (Thermo-Forma, Marietta, OH). Alternatively, an equivalent volume of RPMI was added for the negative control treatments. Immediately after the preincubation period, 2′,7′-dichlorofluorescein diacetate assay (0.2 mg/mL) was added (125 μL), and samples were then mixed and aliquoted (8 replicates per sample) into a clear 96-well flat-bottomed plate. Oxidative

burst was measured (excitation = 485; emission = 530) every 15 min for 75 min at 42°C in the fluorescent plate reader.

Serum from each bird was collected and stored at −20°C until assayed. Clinical chemistry analysis of serum levels of creatine kinase (**CK**), alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), lactate dehydrogenase (**LDH**), alkaline phosphatase (**AP**), glucose, cholesterol, uric acid, blood urea nitrogen, phosphorus, triglycerides, calcium, and iron were determined for each bird using an Express Plus automated clinical chemistry analyzer (Ciba-Corning Diagnostics Corp., Medfield, MA).

Transport swabs were used to culture the air sac and liver of each bird and were immediately taken to the laboratory where they were plated on MacConkey agar. Representative lactose-negative colonies were identified using API 20-E test kits (Bio-Mérieux Vitek Inc., Hazelwood, MO) and were compared with the challenge strain. Tissues having isolation of lactose-negative colonies with the same API 20-E profile as the challenge strain were considered positive.

Statistics

Data were analyzed by pen as a 3 × 2 factorial arrangement (3 feed treatments × 2 challenges) or as a 2 × 2 factorial for HET oxidative burst, using the GLM procedure of SAS software for ANOVA. Means were separated using the least squares means procedure with pen as the experimental unit (SAS Institute, 1999). A P-value of less than 0.05 was considered significant unless otherwise stated.

RESULTS

The main effect mean (**MEM**) wk 3 BW was significantly decreased by the stress challenge ($P = 0.01$) and was significantly higher in control birds fed 1,000 g/ton of YE as compared with stressed birds fed 1,000 g/ton of YE ($P = 0.02$, Figure 1). There was no diet × stress interaction.

The total leukocyte counts and the percentage of LYM were decreased and the percentages of HET and the HET:LYM ratio were increased by stress challenge of control-fed birds; however, there were no effects of the stress challenge in either group of YE-treated birds (Table 1). Yeast extract treatment significantly increased the MEM for percentage HET, Het:Lym ratio, hematocrit, and hemoglobin. There was a significant diet × stress interaction affecting percentage of HET, percentage of LYM, percentage of MONO, and LYM numbers (data not shown). Percentage of HET and the HET:LYM ratio were increased and percentage of LYM was decreased in nonstressed birds by both YE treatments relative to control-fed birds; however, there was no further increase due to stress in YE-treated birds. Percentage of HET in stressed birds was decreased in birds fed 1,000 g/ton of YE relative to those fed 500 g/ton (Table 1). The MEM for HET:LYM ratio was increased and this was the only MEM for blood cell or hematology parameters that was affected by the stress challenge (Table 1).

The numbers of HET in whole blood were significantly higher in nonstressed control birds fed 1,000 g/ton of YE as compared with nonstressed birds fed control feed (Figure 2). The MEM for HET oxidative burst activity was increased by time ($P < 0.0001$), stress ($P < 0.0001$), and 1,000 g/ton of YE supplementation ($P < 0.0001$). All treatments were significantly different from each other after the initial 15-min observation. Yeast extract supplementation of nonstressed birds (treatment 2) significantly increased oxidative burst activity relative to nonsupplemented, nonstressed birds (treatment 1) and YE supplementation of stressed birds (treatment 4) significantly decreased activity relative to stressed control-fed birds (treatment 3) (Figure 3).

Yeast extract increased the MEM for serum levels of uric acid; however, this effect was only seen in stressed birds fed 1,000 g/ton of YE (Table 2). The MEM for

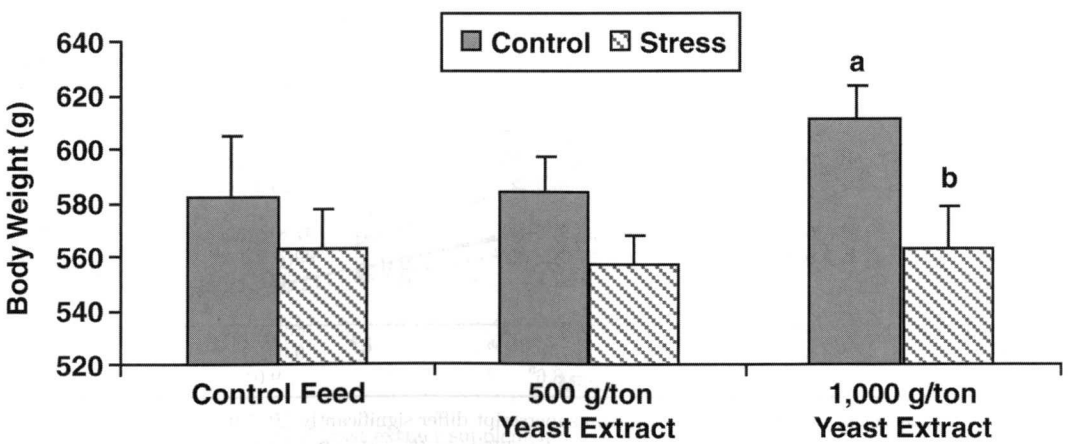

Figure 1. Effects of stress and 500 or 1,000 g/ton of yeast extract supplementation on average BW of 3-wk-old turkey poults. Means with no common letters are significantly different ($P < 0.05$). Main effect mean for stress, $P = 0.01$. No interaction. Color version available in the online PDF.

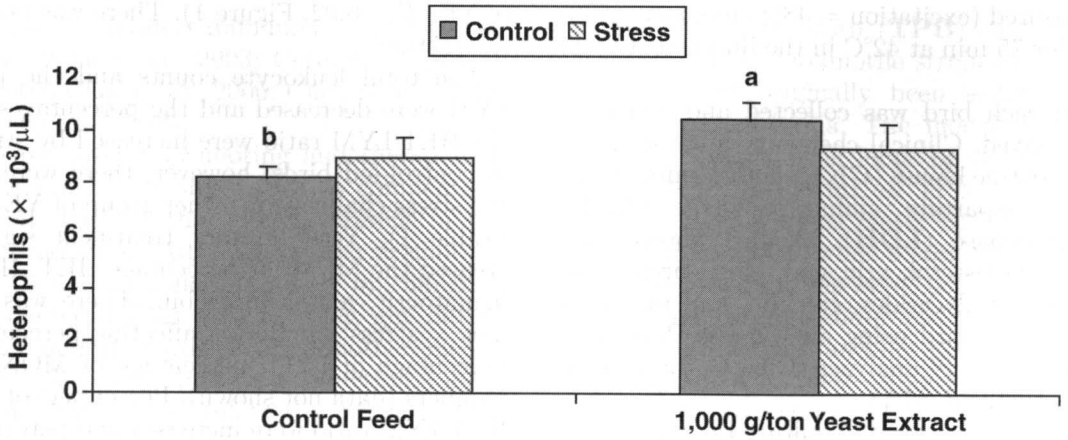

Figure 2. Effects of stress and 1,000 g/ton of yeast extract supplementation on numbers of heterophils in peripheral blood. Means with no common letters are significantly different ($P = 0.05$). Color version available in the online PDF.

phosphorus, triglycerides, and calcium were decreased by YE treatment and this effect was only significant in the 500 g/ton treatment. Yeast extract tended to decrease cholesterol levels in nonstressed birds and increase levels in stressed birds and levels were significantly decreased by 500 g/ton of YE in nonstressed birds (Table 2). Stress challenge increased the MEM serum levels of glucose, total protein, cholesterol, blood urea nitrogen, uric acid, phosphorus, and triglycerides and decreased levels of iron and calcium (Table 2). Lev-

Table 1. Effect of yeast extract (Alphamune, Alpharma Animal Health, Antwerp, Belgium) supplementation and stress challenge[1] on total leukocyte counts (WBC); percentage of heterophils (HET), lymphocytes (LYM), monocytes (MONO), and basophils (BASO); HET:LYM ratio; red blood cell counts, hematocrit (HCT), and hemoglobin concentration (HGB)

Variable	Treatment	Control feed	500 g/ton of yeast extract	1,000 g/ton of yeast extract	Stress MEM[2]	*P*-value
WBC						
($\times 10^3/\mu L$)	Control	19.1 ± 1.5^{x}	16.8 ± 0.8	17.5 ± 1.5	17.8	0.20
Diet MEM	Stress	15.3 ± 1.4^{y}	18.2 ± 1.9	15.0 ± 1.5	16.2	
	($P = 0.7$)	17.2	17.5	16.3		
HET						
(%)	Control	$45.8 \pm 3.3^{b,y}$	59.3 ± 4.3^{a}	61.7 ± 3.1^{a}	55.6	0.6
Diet MEM	Stress	$59.4 \pm 2.3^{ab,x}$	50.5 ± 7.7^{b}	62.5 ± 3.1^{a}	57.5	
	($P = 0.02$)	52.6^{b}	54.9^{ab}	62.1^{a}		
LYM						
(%)	Control	$39.5 \pm 4.0^{a,x}$	22.6 ± 4.8^{b}	22.3 ± 3.6^{b}	28.1	0.27
Diet MEM	Stress	20.4 ± 2.1^{y}	33.3 ± 9.0	20.3 ± 3.8	24.7	
	($P = 0.08$)	30.0	28.0	21.3		
HET:LYM						
Ratio	Control	$1.5 \pm 0.2^{b,y}$	4.3 ± 1.1^{a}	3.6 ± 0.5^{a}	3.1^{y}	0.04
Diet MEM	Stress	3.6 ± 0.5^{x}	3.7 ± 1.1	5.1 ± 1.0	4.1^{x}	
	($P = 0.02$)	2.5^{b}	4.0^{ab}	4.4^{a}		
MONO						
(%)	Control	14.4 ± 1.0^{y}	17.9 ± 1.7	16.0 ± 0.9	16.1	0.27
Diet MEM	Stress	20.0 ± 1.9^{x}	16.0 ± 2.5	16.9 ± 1.2	17.6	
	($P = 0.9$)	17.2	16.9	16.4		
BASO						
(%)	Control	21.1 ± 1.0^{a}	11.2 ± 1.7^{b}	13.4 ± 0.9^{b}	16.1	0.06
Diet MEM	Stress	20.6 ± 1.9	20.2 ± 2.5	21.3 ± 1.2	17.6	
	($P = 0.1$)	17.2	16.9	16.4		
HCT						
(%)	Control	36.4 ± 0.5	36.9 ± 1.0	37.8 ± 0.6	37.0	0.3
Diet MEM	Stress	36.2 ± 0.5^{b}	37.3 ± 0.8^{b}	39.7 ± 1.2^{a}	37.7	
	($P = 0.005$)	36.3^{b}	37.1^{b}	38.8^{a}		
HGB						
(g/dL)	Control	8.8 ± 0.1^{ab}	8.4 ± 0.2^{b}	8.9 ± 0.1^{a}	8.70	0.7
Diet MEM	Stress	8.4 ± 0.1^{b}	8.7 ± 0.1^{ab}	9.0 ± 0.2^{a}	8.74	
	($P = 0.008$)	8.6^{b}	8.6^{b}	9.0^{a}		

[a,b]Means within a row of each treatment or MEM with no common superscript differ significantly ($P \leq 0.05$).

[x,y]Means within a column of each parameter with no common superscript differ significantly ($P \leq 0.05$).

[1]Stress challenge model consisted of injection of approximately 60 cfu of *Escherichia coli* into the air sac at 1 wk of age followed by a 12-h transport and holding procedure at 3 wk of age. Blood was collected 12 h after the end of the transport period.

[2]Main effect mean.

els of the serum enzymes CK, ALT, AST, and LDH were significantly elevated by the stress challenge and were not affected by YE supplementation (Table 3). There were no differences due to either stress challenge or feed supplementation for serum levels of albumen, magnesium, or AP (data not shown). There were no significant feed × stress interactions for data in Table 2 or Table 3.

The MEM for isolation of the challenge strain of *E. coli* from the liver was decreased by both YE and the stress challenge ($P = 0.05$). The challenge strain of *E. coli* was isolated from the livers of 13.3% of nonstressed birds fed the control diet, 6.9% fed 1,000 g/ton of YE, and 0% fed 500 g/ton of YE. The stress challenge resulted in a significant decrease in bacterial isolation from the liver of control-fed birds as compared with nonchallenged birds. There was no *E. coli* isolated from the livers of any birds provided the 500 g/ton of YE treatment or from stressed birds provided the 1,000 g/ton of YE treatment (Figure 4).

The challenge strain of *E. coli* was isolated from the air sacs of 6.7% of nonstressed birds fed the control diet and 3.4% of stressed birds fed the control diet and nonstressed birds fed 1,000 g/ton of YE. Challenge strain isolation from the air sac was negative for all of the birds fed 500 g/ton of YE and stressed birds fed 1,000 g/ton of YE; however, none of these differences were significant (Figure 5). Generic strains of *E. coli* were isolated from the air sac of significantly more nonstressed birds fed the control diet (16.7%) compared with all other treatments ($P = 0.03$) (Figure 6).

DISCUSSION

Systemic infection with *E. coli* (colibacillosis) is an important poultry disease and is the most frequent cause of airsacculitis leading to condemnation (Barnes et al., 2008). Although airsacculitis has a complex etiology, host susceptibility is thought to be more important than bacterial virulence, and abnormal stress is associated with infection. In the present study, the challenge strain of *E. coli* was isolated from the livers and air sacs of nonchallenged control birds, suggesting that environmental transmission was involved. However, dietary supplementation with YE (Alphamune) increased HET numbers and oxidative burst activity and these changes may explain the decrease in the isolation of *E. coli* from both air sac and liver of birds fed the supplemented diets.

The HET is a highly phagocytic granulated cell capable of antimicrobial activity and is analogous, though not identical, to the mammalian neutrophil (Harmon, 1998). Although activation of HET is an important component of the innate immune response, these cells are also responsible for inflammatory tissue damage (Blüml et al., 2008). In mammals, the rate of neutrophil production can increase 10-fold during stress and infection (Smith, 1994). Human patients with infection have been shown to have a subpopulation of neutrophils with an enhanced oxidative burst and males subjected to moderate exercise had increased percentages of neutrophils that were highly responsive to PMA (Smith, 1994).

In this study, the stress challenge dramatically increased the oxidative burst activity of PMA-stimulated cells. In contrast, YE supplementation moderately increased oxidative burst in the nonstressed control birds and modulated the response of stressed birds. The combination of the stress challenge and YE supplementation completely prevented bacterial colonization of liver and air sac in this study, even though the stressed birds had been inoculated with low levels of *E. coli* at 1 wk of age. The ability of β-glucans to similarly modulate the effects of stress on the oxidative burst activity of fish neutrophils was previously reported (Jeney et al., 1997; Volpatti et al., 1998; Palic et al., 2006).

β-Glucans, as well as β-glucan molecules from other sources, have been shown to increase oxidative burst

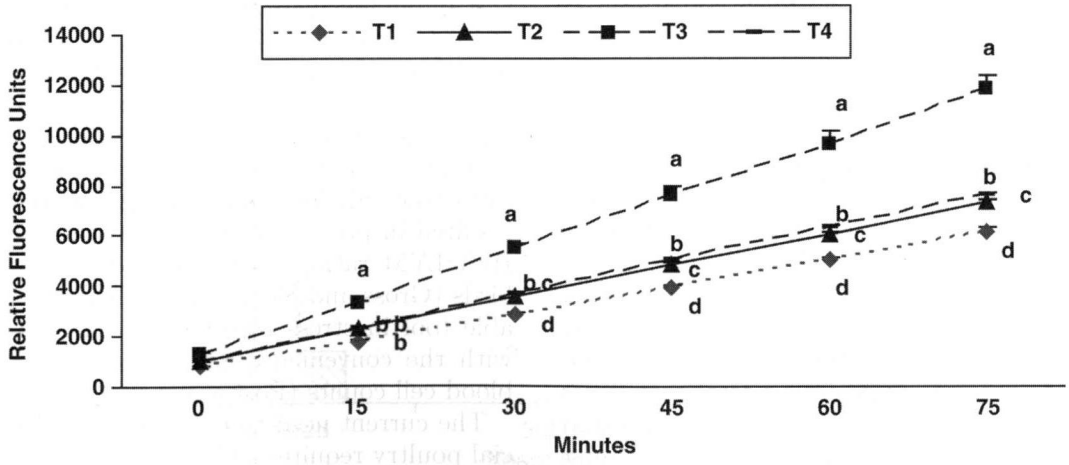

Figure 3. Effects of stress and 1,000 g/ton of yeast extract supplementation on oxidative burst activity of heterophils at 0, 15, 30, 45, 60, and 75 min after 2′,7′-dichlorofluorescein diacetate addition. T = Treatment. T1 = control feed, no stress; T2 = 1,000 g/ton of yeast extract/ton, no stress; T3 = control feed, stress; T4 = 1,000 g/ton of yeast extract/ton, stress. Data are presented as mean and SEM of the relative fluorescence of phorbol myristate acetate-stimulated cells, n = 8. Means with no common letters are significantly different ($P < 0.05$). Color version available in the online PDF.

Table 2. Effects of yeast extract (Alphamune, Alpharma Animal Health, Antwerp, Belgium) supplementation and stress challenge[1] on levels of glucose, total protein, cholesterol, blood urea nitrogen (BUN), iron, uric acid, phosphorus, triglycerides, and calcium in turkey serum

Variable	Treatment	Control feed	500 g/ton of yeast extract	1,000 g/ton of yeast extract	Stress MEM[2]	*P*-value
Glucose						
(mg/dL)	Control	245.9 ± 26.6	259.9 ± 6.6	219.7 ± 26.9^{y}	241.8^{y}	0.01
Diet MEM	Stress	288.3 ± 3.9	270.6 ± 10.4	277.4 ± 6.0^{x}	278.7^{x}	
	($P = 0.5$)	267.1	265.2	248.6		
Total protein						
(g/dL)	Control	2.55 ± 0.05	2.53 ± 0.09^{y}	2.61 ± 0.05	2.56^{y}	0.003
Diet MEM	Stress	2.64 ± 0.04	2.72 ± 0.07^{x}	2.73 ± 0.05	2.70^{x}	
	($P = 0.4$)	2.60	2.63	2.67		
Cholesterol						
(mg/dL)	Control	167.5 ± 7.8^{a}	144.7 ± 6.5^{b,y}	149.5 ± 7.3^{ab,y}	153.9^{y}	0.02
Diet MEM	Stress	159.7 ± 5.6	175.1 ± 8.2^{x}	171.7 ± 7.5^{x}	168.8^{x}	
	($P = 0.9$)	163.6	159.9	160.6		
BUN						
(mg/dL)	Control	1.31 ± 0.1	1.42 ± 0.1	1.27 ± 0.1^{y}	1.33^{y}	0.007
Diet MEM	Stress	1.70 ± 0.2	1.73 ± 0.1	1.77 ± 0.2^{x}	1.73^{x}	
	($P = 0.9$)	1.50	1.58	1.52		
Iron						
(μg/dL)	Control	175.8 ± 11.7	143.6 ± 16.3	167.3 ± 12.5^{x}	162.2^{x}	0.01
Diet MEM	Stress	138.7 ± 15.4	125.3 ± 20.1	121.2 ± 16.5^{y}	128.4^{y}	
	($P = 0.4$)	157.3	134.4	144.2		
Uric acid						
(mg/dL)	Control	2.98 ± 0.3	2.30 ± 0.3	3.39 ± 0.3^{y}	2.89^{y}	0.0005
Diet MEM	Stress	4.49 ± 0.4^{b}	4.04 ± 0.4^{b}	7.06 ± 1.4^{a,x}	5.20^{x}	
	($P = 0.02$)	3.73^{b}	3.17^{b}	5.22^{a}		
Phosphorus						
(mg/dL)	Control	5.97 ± 0.3^{y}	5.38 ± 0.4	5.65 ± 0.2^{y}	5.67^{y}	0.003
Diet MEM	Stress	6.77 ± 0.3^{a,x}	5.64 ± 0.2^{b}	6.83 ± 0.3^{a,x}	6.41^{x}	
	($P = 0.02$)	6.47^{a}	5.51^{b}	6.24^{a}		
Triglyceride						
(mg/dL)	Control	108.7 ± 6.7^{a,y}	74.7 ± 5.3^{b}	102.5 ± 7.7^{ab,y}	95.3^{y}	<0.0001
Diet MEM	Stress	153.7 ± 9.7^{a,x}	100.7 ± 18.5^{b}	138.1 ± 9.7^{a,x}	130.8^{x}	
	($P = 0.0005$)	131.2^{a}	87.7^{b}	120.3^{a}		
Calcium						
(mg/dL)	Control	10.8 ± 0.2	10.3 ± 0.5^{x}	10.8 ± 0.1	10.6^{x}	0.03
Diet MEM	Stress	10.9 ± 0.1^{a}	9.1 ± 0.6^{b,y}	10.2 ± 0.3^{a}	10.1^{y}	
	($P = 0.004$)	10.9^{a}	9.7^{b}	10.5^{a}		

^{a,b}Means within a row of each treatment or MEM with no common superscript differ significantly ($P \leq 0.05$).

^{x,y}Means within a column of each parameter with no common superscript differ significantly ($P \leq 0.05$).

[1]Stress challenge model consisted of injection of approximately 60 cfu of *Escherichia coli* into the air sac at 1 wk of age followed by a 12-h transport and holding procedure at 3 wk of age. Blood was collected 12 h after the end of the transport period.

[2]Main effect mean.

in mammalian and fish neutrophils (Liang et al., 1998; Wakshull et al., 1999; Palic et al., 2006; Sauerwein et al., 2006; Murphy et al., 2007). The oxidative burst in chicken HET has also been found to be upregulated by β-glucan (Lowry et al., 2005) and it has been suggested that avian HET and MONO also express the β-glucan receptor, dectin 1 (Nerren and Kogut, 2009).

Stress is known to have biphasic effects on physiology in mammals, in that both too little and too much stress can be detrimental (Sapolsky, 1997; Dhabhar, 2007). Furthermore, what constitutes too little or too much stress is determined at the individual level by genetics, environment, and prior experience (Biondi and Zannino, 1997). In the present study, a 12-h transport stress protocol tended to decrease BW but also increased both the percentage of HET and their oxidative burst activity, resulting in protection from bacterial colonization of both the liver and air sac relative to nonstressed control birds. In poultry, stress has variable effects on the immune system and can both enhance and suppress responses depending on the type and degree of stress and individual variation in the host response (Siegel, 1995). These data suggest that YE supplementation may in itself function as a moderate stressor that may thus help to modulate the oxidative stress response in turkeys subjected to stress. Heterophil numbers, percentages, oxidative burst activity, and the HET:LYM ratio were all increased by YE supplementation and resulted in protection from bacterial colonization. The HET:LYM ratio is a recognized measure of stress in birds (Gross and Siegel, 1983) that has become a valuable tool in stress research especially when combined with the convenience and repeatability of automated blood cell counts (Post et al., 2003; Huff et al., 2005).

The current need to improve the welfare of commercial poultry requires additional objective criteria to determine the level of stress in poultry at an individual level.

The determination of stress-susceptible individuals may be useful in the genetic selection of turkeys with a

Table 3. Effects of yeast extract (Alphamune, Alpharma Animal Health, Antwerp, Belgium) supplementation and stress challenge[1] on levels of creatine kinase, (CK), alanine aminotransferase (ALT), aspartate aminotrasferase (AST), and lactate dehydrogenase (LDH) in turkey serum

Variable	Treatment	Control feed	500 g/ton of yeast extract	1,000 g/ton of yeast extract	Stress MEM[2]	*P*-value
CK						
(U/L)	Control	1,198 ± 99^{y}	1,059 ± 93^{y}	1,223 ± 86^{y}	1,160^{y}	
Diet MEM	Stress	2,582 ± 259^{x}	2,411 ± 169^{x}	2,540 ± 235^{x}	2,511^{x}	<0.0001
	($P = 0.7$)	1,890	1,735	1,882		
ALT						
(U/L)	Control	4.61 ± 0.3	4.60 ± 0.4^{y}	5.09 ± 0.6^{y}	4.76^{y}	
Diet MEM	Stress	5.73 ± 0.5	6.46 ± 0.5^{x}	6.56 ± 0.5^{x}	6.25^{x}	0.0006
	($P = 0.4$)	5.17	5.23	5.82		
AST						
(U/L)	Control	262.6 ± 8.3^{y}	259.7 ± 13^{y}	271.7 ± 7.6^{y}	264.7^{y}	
Diet MEM	Stress	326.7 ± 7.7^{x}	326.3 ± 8.5^{x}	335.2 ± 11.3^{x}	329.4^{x}	<0.0001
	($P = 0.5$)	294.7	293.0	303.5		
LDH						
(U/L)	Control	854.7 ± 39^{y}	845.8 ± 48	938.7 ± 24	879.7^{y}	
Diet MEM	Stress	1,073.8 ± 58^{x}	1,012.7 ± 97	1,076. ± 73	1,054.2^{x}	0.0007
	($P = 0.4$)	964.2	929.2	1,007.4		

^{x,y}Means within a column of each parameter with no common superscript differ significantly ($P \leq 0.05$).

[1]Stress challenge model consisted of injection of approximately 60 cfu of *Escherichia coli* into the air sac at 1 wk of age followed by a 12-h transport and holding procedure at 3 wk of age. Blood was collected 12 h after the end of the transport period.

[2]Main effect mean.

moderate response to the stressors of commercial turkey production.

We have previously reported that increases in the serum levels of CK, ALT, and AST and decreases in alkaline phospatase and iron seen in 15-wk-old turkeys subjected to transport stress may be useful for profiling individuals and flocks to determine their responses to transport stress and feed withdrawal and possibly more general stress responses (Huff et al., 2008). In the present study of much younger birds, the response to transport stress involved increases in CK, ALT, AST, and LDH and decreases in iron with no affect on AP. A dramatic affect in these younger birds was a highly significant increase ($P < 0.0001$) in serum triglyceride levels. The increase in triglyceride levels seen in stressed birds in the present study supports the observations seen in stress models using adrenocorticotropic hormone infusion (Latour et al., 1996; Puvadolpirod and Thaxton, 2000) and in turkeys undergoing the stressors of crowding and overheating (Kowalski et al., 2002). In the present study, the increase appears to have been modulated by YE supplementation, which significantly decreased triglyceride levels, particularly at the 500 g/ton level.

Uric acid is a major antioxidant in birds and has been used as a biomarker for physiological processes such as oxidative stress and tubular function (Hartman

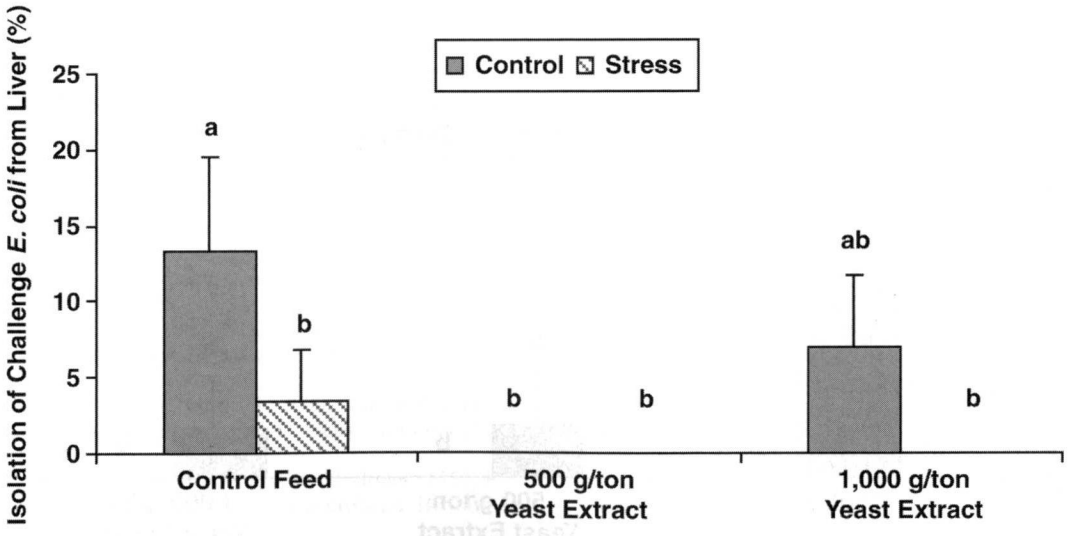

Figure 4. Effects of stress and 500 or 1,000 g/ton of Alphamune (Alpharma Animal Health, Antwerp, Belgium) supplementation on isolation of the challenge strain of *Escherichia coli* from liver. Means with no common letters are significantly different ($P < 0.05$). Main effect mean for diet, $P = 0.05$. Main effect mean for stress, $P = 0.05$. No interaction. Color version available in the online PDF.

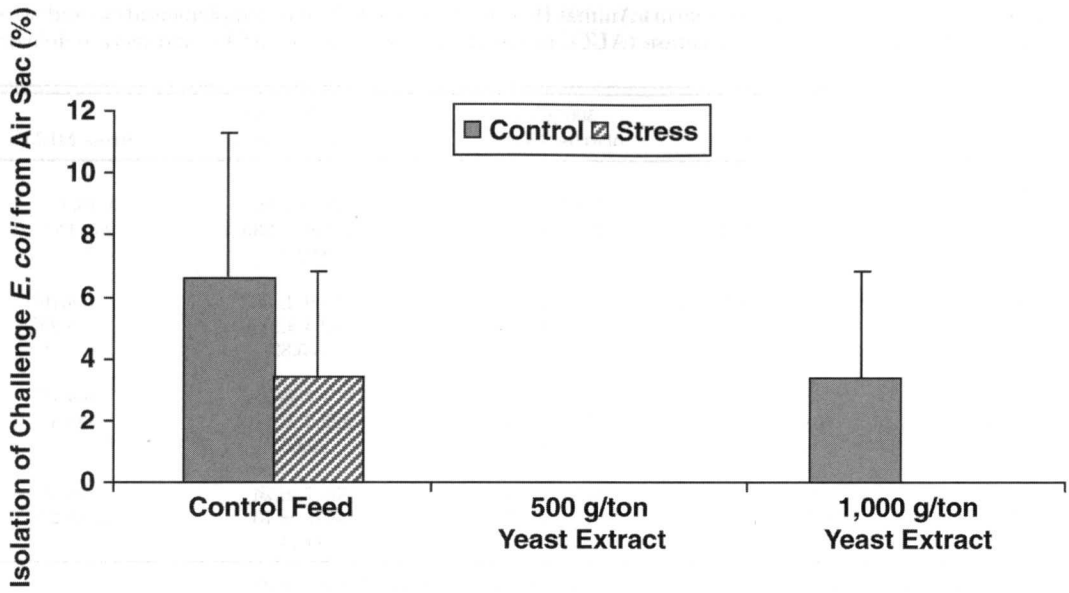

Figure 5. Effects of stress and 500 or 1,000 g/ton of Alphamune (Alpharma Animal Health, Antwerp, Belgium) supplementation on isolation of the challenge strain of *Escherichia coli* from air sac. Differences are not significant. Color version available in the online PDF.

et al., 2006). In this study, both the stress challenge and 1,000 g/ton of YE supplementation increased serum uric acid levels.

We have previously reported that uric acid levels were increased by transport stress (Huff et al., 2008), in agreement with a previous report in which transport stress was found to increase uric acid levels in 3 out of 7 broiler flocks (Halliday et al., 1977).

In the present study, CK levels were increased by transport stress but were not affected by yeast supplementation. Creatine kinase has been suggested as a marker for the degree of stress susceptibility in pigs, with the suggestion that the stress resistance of some genetic lines is reflected by the degree of muscle cell membrane permeability (Reddy et al., 1971). In turkeys, CK levels increase with age and are higher in fast-growing strains (Hocking et al., 1998). The changes seen in CK activity in wild turkeys after capture were correlated with mortality at 14 d postcapture, suggesting that susceptible individuals could be identified by their CK response (Nicholson et al., 2000).

In summary, YE supplementation of turkey poults was shown to increase HET percentages and oxidative burst activity and these changes were correlated with a decrease in the isolation of *E. coli* from both air sac and liver. In addition, YE modulated the dramatic increase in oxidative burst and serum triglycerides due to transport stress. The immunomodulating ability of YE

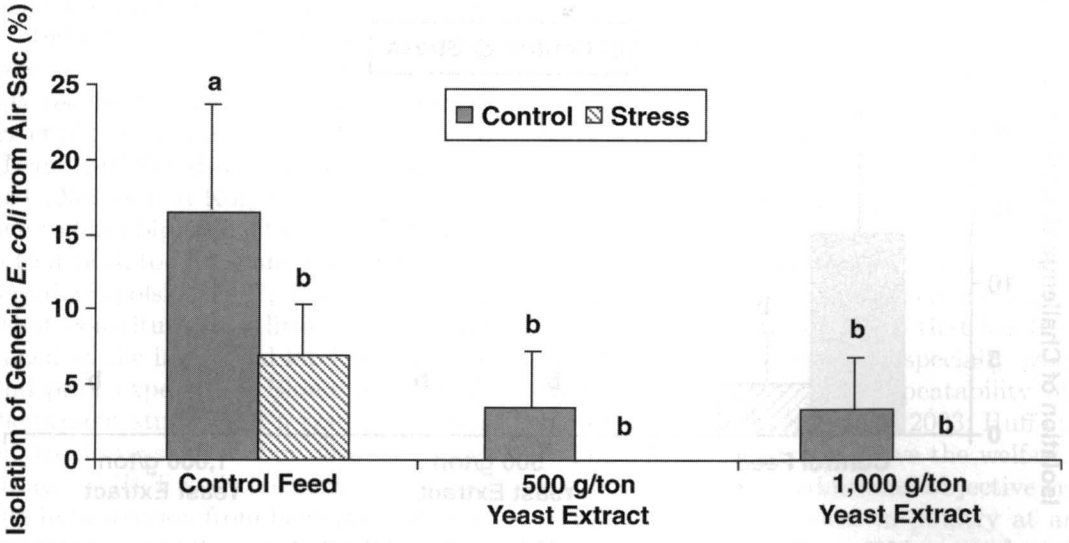

Figure 6. Effects of stress and 500 or 1,000 g/ton of Alphamune (Alpharma Animal Health, Antwerp, Belgium) supplementation on isolation of generic *Escherichia coli* from air sac. Means with no common letters are significantly different ($P < 0.05$). Main effect mean for diet, $P = 0.03$. Main effect mean for stress, $P = 0.03$. No interaction. Color version available in the online PDF.

supports its development as an alternative to antibiotic use in turkey production.

ACKNOWLEDGMENTS

We are grateful to Steven Clark and Kurt Dobson, Alpharma Animal Health, for providing Alphamune for these studies and also for their advice and assistance in this project. We also acknowledge the excellent technical assistance of Dana Bassi, Sonia Tsai, Scott Zornes, David Horlick, and Wally McDonner (USDA, Agricultural Research Service, Poultry Production and Product Safety Research Unit).

REFERENCES

Barnes, J. H., L. K. Nolan, and J. P. Vaillancourt. 2008. Colibacillosis. Pages 691–732 in Diseases of Poultry. 12th ed. Y. M. Saif, ed. Blackwell Publishing, Ames, IA.

Biondi, M., and L. G. Zannino. 1997. Psychological stress, neuroimmunomodulation, and susceptibility to infectious diseases in animals and man: A review. Psychother. Psychosom. 66:3–26.

Blüml, S., B. Rosc, A. Lorincz, M. Seyerl, S. Kirchberger, O. Oškolkova, V. N. Bochkov, O. Majdic, E. Ligeti, and J. Stöckl. 2008. The oxidative state of phospholipids controls the oxidative burst in neutrophil granulocytes. J. Immunol. 181:4347–4353.

Bradley, G. L., T. F. Savage, and K. I. Timm. 1994. The effects of supplementing diets with *Saccharomyces cerevisiae* var. *boulardii* on male poult performance and ileal morphology. Poult. Sci. 73:66–70.

Cetin, N., B. K. Guclu, and E. Cetin. 2005. The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys. J. Vet. Med. A Physiol. Pathol. Clin. Med. 52:263–267.

Chae, B. J., J. D. Lohakare, W. K. Moon, S. L. Lee, Y. H. Park, and T. W. Hahn. 2006. Effects of supplementation of β-glucan on the growth performance and immunity in broilers. Res. Vet. Sci. 80:291–298.

Chen, K. L., B. C. Weng, M. T. Chang, Y. H. Liar, T. T. Chen, and C. Chu. 2008. Direct enhancement of the phagocytic and bactericidal capability of abdominal macrophage of chicks by β-1,3–1,6-glucan. Poult. Sci. 87:2242–2249.

Dhabhar, F. S. 2007. Immune function, stress-induced enhancement. Pages 455–461 in Encyclopedia of Stress. Vol. 2. 2nd ed. G. Fink, ed. Academic Press, London, UK.

Dibner, J. J., and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci. 84:634–643.

Farnell, M. B., A. M. Donoghue, F. Solis De Los Santos, P. J. Blore, B. M. Hargis, G. Tellez, and D. J. Donoghue. 2006. Upregulation of oxidative burst and degranulation in chicken heterophils stimulated with probiotic bacteria. Poult. Sci. 85:1900–1906.

Gomez-Verduzco, G., A. Cortes-Cuevas, C. Lopez-Coello, E. Avila-Gonzalea, and G. M. Nava. 2009. Dietary supplementation of mannan-oligosaccharide enhances neonatal immune responses in chickens during natural exposure to *Eimeria* spp. Acta Vet. Scand. 51:11.

Gross, W. B., and H. S. Siegel. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27:972–979.

Guo, Y., R. A. Ali, and M. A. Qureshi. 2003. The influence of β-glucan on immune responses in broiler chicks. Immunopharmacol. Immunotoxicol. 25:461–472.

Halliday, W. G., J. G. Ross, G. Christie, and R. M. Jones. 1977. Effect of transportation on blood metabolites in broilers. Br. Poult. Sci. 18:657–659.

Harmon, B. G. 1998. Avian heterophils in inflammation and disease resistance. Poult. Sci. 77:972–977.

Hartman, S., S. A. Taleb, T. Geng, K. Gyenai, X. Guan, and E. Smith. 2006. Comparison of plasma uric acid levels in five varieties of the domestic turkey, *Meleagris gallopavo*. Poult. Sci. 85:1791–1794.

Hocking, P. M., M. A. Mitchell, R. Bernard, and D. A. Sandercock. 1998. Interaction of age, strain, sex, and food restriction on plasma creatine kinase activity in turkeys. Br. Poult. Sci. 39:360–364.

Hooge, D. M. 2004a. Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide, 1993–2003. Int. J. Poult. Sci. 3:163–174.

Hooge, D. M. 2004b. Turkey pen trials with dietary mannan oligosaccharide: Meta-analysis, 1993–2003. Int. J. Poult. Sci. 3:179–188.

Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath. 2000. The effect of vitamin D_3 on resistance to stress-related infection in an experimental model of turkey osteomyelitis complex. Poult. Sci. 79:672–679.

Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath. 2004. The effects of water supplementation with vitamin E and sodium salicylate (Uni-Sol®) on the resistance of turkeys to *Escherichia coli* respiratory infection. Avian Dis. 48:324–331.

Huff, G. R., W. E. Huff, J. M. Balog, N. C. Rath, N. B. Anthony, and K. E. Nestor. 2005. Stress response differences and disease susceptibility reflected by heterophil to lymphocyte ratio in turkeys selected for increased body weight. Poult. Sci. 84:709–717.

Huff, G. R., W. E. Huff, N. C. Rath, F. Solis de los Santos, M. B. Farnell, and A. M. Donoghue. 2007. Influence of hen age on the response of turkey poults to cold stress, *Escherichia coli* challenge, and treatment with a yeast extract antibiotic alternative. Poult. Sci. 86:636–664.

Huff, G. R., W. E. Huff, N. C. Rath, N. B. Anthony, and K. E. Nestor. 2008. Effects of *Escherichia coli* challenge and transport stress on hematology and serum chemistry values of three genetic lines of turkeys. Poult. Sci. 87:2234–2241.

Huff, G. R., W. E. Huff, N. C. Rath, and G. Tellez. 2006. Limited treatment with β-1,3/1,6-glucan improves production values of broiler chickens challenged with *Escherichia coli*. Poult. Sci. 85:613–618.

Jeney, G., M. Galeotti, D. Volpatti, Z. Jeney, and D. P. Anderson. 1997. Prevention of stress in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different doses of glucan. Aquaculture 154:1–15.

Kowalski, A., P. Mormede, K. Jakubowski, and M. Jedlinska-Krakowska. 2002. Comparison of susceptibility to stress in two genetic lines of turkey broilers BUT-9 and Big-6. Pol. J. Vet. Sci. 5:145–150.

Latour, M. A., S. A. Laiche, J. R. Thompson, A. L. Pond, and E. D. Peebles. 1996. Continuous infusion of adrenocorticotropin elevates circulating lipoprotein cholesterol and corticosterone concentrations in chickens. Poult. Sci. 75:1428–1432.

Leung, M. Y. K., C. Liu, J. C. M. Koon, and K. P. Fung. 2006. Polysaccharide biological response modifiers. Immunol. Lett. 105:101–114.

Liang, J., D. Melican, L. Cafro, G. Palace, L. Fisette, R. Armstrong, and M. L. Patchen. 1998. Enhanced clearance of a multiple antibiotic resistant *Staphylococcus aureus* in rats treated with PGG-glucan is associated with increased leukocyte counts and increased neutrophil oxidative burst activity. Int. J. Immunopharmacol. 20:595–614.

Lowry, V. K., M. B. Farnell, P. J. Ferro, C. L. Swaggerty, A. Bahl, and M. H. Kogut. 2005. Purified β-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar Enteritidis. Int. J. Food Microbiol. 98:309–318.

Morales-Lopez, R., E. Auclair, F. Garcia, E. Esteve-Garcia, and J. Brufau. 2009. Use of yeast cell walls; β-1,3/1,6-glucans; and mannoproteins in broiler chicken diets. Poult. Sci. 88:601–607.

Murphy, E. A., J. M. Davis, A. S. Brown, M. D. Carmichael, A. Ghaffar, and E. P. Mayer. 2007. Oat β-glucan effects on neutrophil respiratory burst activity following exercise. Med. Sci. Sports Exerc. 39:639–644.

Nerren, J. R., and M. H. Kogut. 2009. The selective Dectin-1 agonist, curdlan, induces an oxidative burst response in chicken heterophils and peripheral blood mononuclear cells. Vet. Immunol. Immunopathol. 127:162–166.

Nicholson, D. S., R. L. Lochmiller, M. D. Stewart, R. E. Masters, and D. M. Leslie Jr. 2000. Risk factors associated with capture-related death in eastern wild turkey hens. J. Wildl. Dis. 36:308–315.

Novak, M., and V. Vetvicka. 2009. Glucans as biological response modifiers. Endocr. Metab. Immune Disord. Drug Targets 9:67–75.

NRC. 1994. Nutrient Requirements of Poultry. National Academy Press, Washington, DC.

Palic, D., C. B. Andreasen, D. M. Herolt, B. W. Menzel, and J. A. Roth. 2006. Immunomodulatory effects of β-glucan on neutrophil function in fathead minnows (*Pimephales promelas* Rafinesque, 1820). Dev. Comp. Immunol. 30:817–830.

Post, J., J. M. J. Rebel, and A. A. H. M. ter Huurne. 2003. Automated blood cell count: A sensitive and reliable method to study corticosterone-related stress in broilers. Poult. Sci. 82:591–595.

Puvadolpirod, S., and J. P. Thaxton. 2000. Model of physiological stress in chickens. 1. Response parameters. Poult. Sci. 79:363–369.

Reddy, M. V. V., L. L. Kastenschmidt, R. G. Cassens, and E. J. Briskey. 1971. Studies on stress susceptibility: The relationship between serum enzyme changes and the degree of stress susceptibility. Life Sci. 10:1381–1391.

Rosen, G. D. 2007a. Holo-analysis of the efficacy of Bio-Mos in broiler nutrition. Br. Poult. Sci. 48:21–26.

Rosen, G. D. 2007b. Holo-analysis of the efficacy of Bio-Mos in turkey nutrition. Br. Poult. Sci. 48:27–32.

Roura, E., J. Homedes, and K. C. Klasing. 1992. Prevention of immunologic stress contributes to the growth-permitting ability of dietary antibiotics in chicks. J. Nutr. 122:2383–2390.

Sapolsky, R. M. 1997. McEwen-induced modulation of endocrine history: A partial review. Stress 2:1–12.

SAS Institute. 1999. SAS/STAT User's Guide: Version 8. SAS Institute Inc., Cary, NC.

Sauerwein, H., S. Schmitz, and S. Hiss. 2006. Effects of a dietary application of a yeast cell wall extract on innate and acquired immunity, on oxidative status and growth performance in weanling pigs and on the ileal epithelium in fattened pigs. J. Anim. Physiol. Anim. Nutr. (Berl.) 91:369–380.

Siegel, H. S. 1995. Stress, strains, and resistance. Br. Poult. Sci. 36:3–22.

Sims, M. D., K. A. Dawson, K. E. Newman, P. Spring, and D. M. Hoogell. 2004. Effects of dietary mannan oligosaccharide, bacitracin methylene disalicylate, or both on the live performance and intestinal microbiology of turkeys. Poult. Sci. 83:1148–1154.

Smith, J. A. 1994. Neutrophils, host defense, and inflammation: A double-edged sword. J. Leukoc. Biol. 56:672–686.

Solis de los Santos, F., A. M. Donoghue, M. B. Farnell, G. R. Huff, W. E. Huff, and D. J. Donoghue. 2007. Gastrointestinal maturation is accelerated in turkey poults supplemented with a mannan-oligosaccharide yeast extract (Alphamune). Poult. Sci. 86:921–930.

Soltanian, S., E. Stuyven, E. Cox, P. Sorgeloos, and P. Bossier. 2009. β-Glucans as immunostimulant in vertebrates and invertebrates. Crit. Rev. Microbiol. 35:109–138.

Stone, C. W. 1998. Yeast Products in the Feed Industry. A Practical Guide for Feed Professionals. http://www.diamondv.com/articles/booklet/booklet.html Accessed Dec. 3, 2009.

Volman, J. J., J. D. Ramakers, and J. Plat. 2008. Dietary modulation of immune function by β -glucan. Physiol. Behav. 94:276–284.

Volpatti, D., L. D'Angelo, G. Jeney, Z. Jeney, D. P. Anderson, and M. Galeotti. 1998. Nonspecific immune response in fish fed glucan diets prior to induced transport stress. J. Appl. Ichthyology 14:201–206.

Wakshull, E., D. Brunke-Reese, J. Lindermuth, L. Fisette, R. S. Nathans, J. J. Crowley, J. C. Tufts, J. Zimmerman, W. Mackin, and D. S. Adams. 1999. PGG-Glucan, a soluble β-(1,3)-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NF-κ B-like factor in human PMN: Evidence for a glycosphingolipid β-(1,3)-glucan receptor. Immunopharmacology 41:89–107.

Xie, H., G. R. Huff, W. E. Huff, J. M. Balog, and N. C. Rath. 2002. Effects of ovotransferrin on chicken macrophages and heterophil-granulocytes. Dev. Comp. Immunol. 26:805–815.

Yang, Y., P. A. Iji, and M. Choct. 2009. Dietary modulation of gut microflora in broiler chickens: A review of the role of six kinds of alternatives to in-feed antibiotics. World's Poult. Sci. J. 65:97–114.

Yang, Y., P. A. Iji, A. Kocher, L. L. Mikkelsen, and M. Choct. 2008a. Effects of mannanoligosaccharide and fructooligosaccharide on the response of broilers to pathogenic *Escherichia coli* challenge. Br. Poult. Sci. 49:550–559.

Yang, Y., P. A. Iji, A. Kocher, E. Thomson, L. L. Mikkelsen, and M. Choct. 2008b. Effects of mannanoligosaccharide in broiler chicken diets on growth performance, energy utilization, nutrient digestibility, and intestinal microflora. Br. Poult. Sci. 49:186–194.

Zdunczyk, Z., J. Juskiewicz, J. Jankowski, E. Biedrzycka, and A. Koncicki. 2005. Metabolic response of the gastrointestinal tract of turkeys to diets with different levels of mannan-oligosaccharide. Poult. Sci. 84:903–909.

Zdunczyk, Z., J. Juskiewicz, J. Jankowski, and A. Koncicki. 2004. Performance and caecal adaptation of turkeys to diets without or with antibiotic and with different levels of mannan-oligosaccharide. Arch. Anim. Nutr. 58:367–378.

Zhang, A. W., B. D. Lee, S. K. Lee, K. W. Lee, G. H. An, K. B. Song, and C. H. Lee. 2005. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poult. Sci. 84:1015–1021.